(NEW SERIES)

No. 15.

SCIENTIFIC MEMOIRS

BY

OFFICERS OF THE MEDICAL AND SANITARY DEPARTMENTS

OF THE

GOVERNMENT OF INDIA.

ON A PARASITE FOUND IN PERSONS SUFFERING FROM ENLARGEMENT OF THE SPLEEN IN INDIA.—(THIRD REPORT.)

BY

LIEUT. S. R. CHRISTOPHERS, M.B., I.M.S.

ISSUED UNDER THE AUTHORITY OF THE GOVERNMENT OF INDIA BY THE SANITARY COMMISSIONER WITH THE GOVERNMENT OF INDIA, SIMLA.

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On a Parasite found in Plasmodium malariæ from Enlargement of the Spleen in India.—(Third Report.)

BY

LIEUT.-COL. E. D. CUNNINGHAM, M.D., I.M.S.

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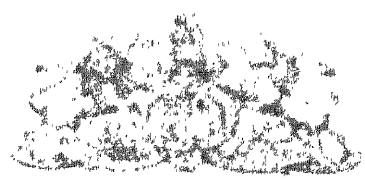

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On a Parasite found in Persons suffering from Enlargement of the Spleen in India.—(Third Report)

The development undergone by the parasite in citrated blood at a low temperature.

THE correct zoological position of the parasites described in my previous reports is extremely uncertain. They have been variously classed as *Inoplasmata*—the view held by Laveran and Mesnil and by Major Donovan, I.M.S.; as *Trypanosomata*—the view of Major Leishman, R.A.M.C. and of Marchand and Ledingham, as protozoa of an unknown nature—the view first put forward by Major Ross, I.M.S., and later taken up by others and as the spores of a microsporidian.

Captain Rogers, I.M.S., recently announced that, by adding blood, obtained by splenic puncture from cases of splenomegaly caused by the parasites, to a small quantity of sterile citrate of sodium solution and keeping it at a temperature of 22°C. for two or three days he had succeeded in observing the development of the parasites into trypanosomes.* He found that, when the citrated splenic blood was kept at blood heat, the parasites very quickly disappeared, but when kept in a cold incubator at 27°C., they retained their usual shape and characters for several days. Moreover, they increased markedly in numbers, and numerous dividing forms were present. At a temperature of 37°C. the parasites lived for only three or four days, and he therefore reduced the temperature of the incubator to 22°C. This temperature was found to be most suitable for the development of the parasites, and he reported that in the cultures obtained from two cases "unmistakeable trypanosoma" appeared, together with smaller pear shaped flagellated bodies and other intermediate forms. "The most marked of the cases showed completely developed trypanosoma with thick flagella, macro-nucleus and micro-nucleus, after incubation for one day, while the living forms were also seen in the blood culture, moving rapidly among the corpuscles" . . . . . "The other case in which the trypanosoma have developed was a case of *kala azar* from Assam, and after incubating for five days at 22°C., we found in the citrated blood a number of intermediate forms and a few fully developed trypanosoma."*

Observers who had put forward the opinion that the bodies are a stage in the development of a trypanosome looked upon Captain Rogers' discovery as a

* *British Medical Journal*, September 17th, 1904, page 632.

conferences of these with their colony, but it was apparent from an examination of the illustrations attached to his article that the bodies which Captain Rogers regarded as fully-developed trypanosomes differed considerably from ordinary trypanosomes found in animals. They possessed no undulating membrane, the micronucleus or centrosome was situated at the end of the parasite from which the flagellum emerged, and the flagellum appeared to pass out of the parasite entirely free from the protoplasm. In a later communication* Captain Rogers emphasised these differences, and stated definitely that in no instance was an undulating membrane present, and that the flagellum pass forwards towards the [illegible] extremity.

So far as I am aware, no other observer than Captain Rogers has yet recorded the extracorporeal development of the bodies, and it is therefore advisable to summarise the main facts noted by him. His procedure appears to be very simple, the following being described as follows:—

"The blood obtained by spinal puncture was immediately ejected into small sterile centrifuge tubes containing a little sodium citrate to prevent the blood from coagulating, and these were then incubated at varying temperatures, portions of the culture being removed with a platinum loop from time to time for examination with the microscope."

From a personal communication I learned that the strength of the solution of sodium citrate used was 2·5 per cent.

The steps of development are noted by Captain Rogers* as follows:—

(a) Development at 37° C.—"The division bodies which appear in great numbers in cultures at 37° C. are of two kinds, the first a simple subdivision of the original parasite into two, the nucleus and the micronucleus first dividing, and then the body of the cell splitting up into the cleavage segments of one sort or the other, as before they separate they remain only attached by the other poles."

"These forms can be found in small numbers in long search in films of blood obtained by spinal puncture, but numerous parasites are present, but they form only a very small proportion of the total number of organisms seen. On the other hand, in cultures they are present in very much larger numbers, several in various stages being often seen in a single field of the microscope."

"The second mode of division is a multiple one. The macro- and micronuclei divide a number of times instead of only once, the outline of the cell becomes less definite, until eventually the appearance is reached in which a number of very small nuclei arranged in pairs of a small and large kind enclosed in a cuticular material is seen. Next these enlarge gradually and each pair become surrounded by a faint capsule, which becomes more and more distinct

* Quarterly Journal of Microscopical Science, November 1904.

with the growth of each young form until the characteristic groups of the oval binucleated fully grown spleen parasite result, which are not very rarely seen in good specimens of spleen puncture blood."

(*c*) *Development at 22°C.*—"On next reducing the temperature of the cold incubator down to about 22°C., and making further cultures in a new series of cases of citrated spleen blood, further and more important changes were soon found."

The various stages of development at 22°C. are given as follows:—

Stage of development after 24 hours.—"At the end of one day at 22°C. the organisms have already increased considerably in size, while the macro-nucleus is also larger, this being a striking feature. On the other hand, the micro-nucleus has not altered, but still remains small and rod shaped. The forms shown in line VII* also show that the macro-nucleus, in addition to being larger, is beginning to present a granular appearance, while it does not stain so darkly as in the original spleen parasites. Further, the protoplasm of the cell is also increasing in amount and now takes on a bluish staining, and has a very finely granular appearance. These are the only changes met with as a rule on the first day."

Stage of development after 48 hours.—"By the end of the second day much more marked changes are met with. In the first place, there is a still further and very marked increase in the size of the organism, still affecting especially the macro-nucleus and the protoplasm. Secondly, and of much greater interest is the appearance of double forms such as are not met with on the first day. These show every degree from apposition at one point of their circumference of two of the large oval forms through closer and closer degrees of contact up to nearly complete fusion of the two cells. At first I took these stages for a method of division, but as a further study showed that the later development into elongate and flagellate forms always takes place in pairs or rarely threes, I have come to the conclusion that these early double forms are really a kind of conjugation, such as is known to occur in other protozoa preparatory to the evolution of new stages in their life history."

Stage of development after 72 hours.—"The third day is characterized by the elongation of the conjugating pair of organisms and the first appearance of flagellate forms, though sometimes the latter may not be found until the fourth day. The commonest appearance of these pyriform bodies is that in which the micro-nuclei have passed to the thinner ends from which the flagella will eventually arise." . . . "In case 47 some early flagellate forms were found on the third day." . . . . "The remaining forms shown in line IX have all reached the elongate stage, although still without flagella."

Stage of development after 96 hours.—"In the figures of line X are shown

* These references are to the plate which illustrates Captain Rogers' article in the *Quarterly Journal of Microscopical Science*.

some of the flagellated forms found on the fourth day in case 47, in addition to which there were much more numerous double pyriform organisms without flagella, for only a very small percentage of the conjugating forms eventually reach the flagellate stage under the artificial conditions of the cultures, which must be very far from being as favourable to the development of the organism as the natural conditions in which it takes place, whatever it may be."

With regard to elongate forms, Captain Rogers notes that, though they occurred in several of his cases, yet they did not always appear. He also notes that the extremely long attenuated forms figured by him in his first publication are exceptional, both in that elongation was more marked than usual, and in that this great degree of development had taken place in 24 hours.

In the main I have been able to confirm Captain Rogers' results. Indeed, most of the forms figured by him, including very elongate forms, have occurred in my preparations. In addition I have been able to observe certain further appearances not described by him, which appear to take place at a later date, and to add some details of description. On the other hand, I have been unable to accept his conclusions regarding conjugation of the large oval forms or to be convinced that the nature of the bodies is yet finally settled.

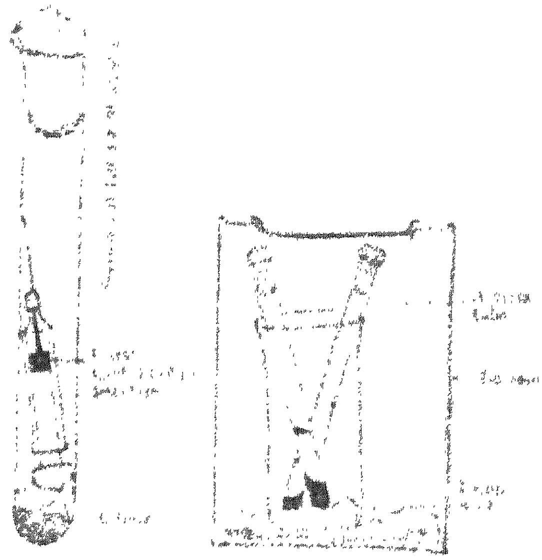
Technique.—At first I made several experiments with weak solutions of citrate of sodium, *i.e.*, from ·5 to 2 per cent. without success, but since I was informed that Captain Rogers used a 10 per cent. solution, I have employed this strength, and it is with this stronger solution that positive results have been obtained. I have found the following the most convenient method of observing the development of oöcysts.

A 10 per cent. solution of citrate of sodium in distilled water is freshly prepared. The salt used in my experiments was obtained by very carefully neutralizing pure sodium hydrate with citric acid. The fluid, after concentration on a water bath, was again neutralized and crystals allowed to form. The crystals were collected and the salt recovered by a second crystallization.

Some time before the operation of spleen puncture is to be carried out, a Burroughs and Wellcome "simplex" syringe (small size) fitted with a platino-iridium needle is rinsed out with the citrate solution and placed in a test tube with the piston resting upon a pad of wool. About ·3 c.c. of citrate solution is left in the syringe and the cotton pad is charged with the same. The test-tube is plugged and the whole sterilized at 120° C. for half an hour in the autoclave.

At the bedside the test-tube is tilted to allow the syringe to fall needle downwards against the plug. The plug is then removed far enough to allow the barrel of the syringe to be seized by the fingers. It is convenient to have three or four prepared syringes at hand, in order to reduce the amount of preparation necessary at the bedside.

Immediately before puncture any excess of fluid is ejected from the syringe, only a little being allowed to remain in the needle and nozzle. A successful puncture should one third to one-half fill the barrel of the syringe.



Illustrating author's method of studying development
of bodies in ejected blood.

Blood from the syringe is ejected with the fullest precautions as to sterility into small sterile tubes. I have found "arsenic tubes" (straight 5" × ⅜") very suitable, their narrowness and length being antagonistic to desiccation and contamination during the frequent examination of the blood. For sterilization these tubes may be heated to 120° C. in the autoclave, or merely passed through the flame till "browning" of the cotton plug takes place. *No citrate solution is added to the tubes.* After blood has been ejected into the tubes, I have found it convenient to enclose them in a small tin canister containing a little damp wool and provided with a tightly fitting lid.

As I was obliged to use an ordinary ice chest, the cultures were exposed to a somewhat varying temperature, but it did not rise above 24°C.

Blood for examination is removed by means of a platinum loop, great care being taken each time to prevent bacterial contamination. As the tubes employed were long and narrow, it was necessary to use a long, rather thick, platinum wire instead of the ordinary loop with a holder.

Before removal of a drop for examination, the blood is shaken up to mix the corpuscles and plasma thoroughly. This enables more satisfactory films to be

made as well as ensures parasites being equally distributed. In good preparations the blood corpuscles remain unchanged for 15 days or more: they cannot be distinguished under the microscope from the corpuscles of freshly drawn blood. The leucocytes show marked degenerative changes, and the nucleus becomes swollen and irregular. Nevertheless in some cases the variety of cell could be distinguished easily after several days.

Development.—Development of the bodies was observed in four cases. In one the preparation was examined every few days for a period of 33 days, at the end of which time the corpuscles were but little altered and no micro-organisms were seen in the films. The remaining cases, owing to the pressure of other duties, were not followed up so systematically, and in two of the cases commencing changes only, though of a convincing nature, were seen. The results noted are as follows:—

Case 1.—The patient had a greatly enlarged spleen reaching across the middle line of the abdomen and an irregular high temperature reaching to 102°F. and 103°F. at night. About ·75 c.c. of blood was removed from the spleen in the manner already described. In films made at the time of puncture parasites were fairly numerous. The specimen was surrounded by ice, so arranged that the preparation should not be made too cold, and carried about six miles; it was then placed in the cool chamber of an ice chest at a temperature varying between 38°F. and 42°F. No clotting occurred, and when shaken up the blood retained its normal appearance.

The first unmistakable sign of development was seen on the third day. On this day, although the majority of the bodies appeared unaltered, yet a considerable number were somewhat larger than usual. A few were also noticed in which the protoplasm had increased in amount and had stained a dark hue. The large chromatin mass had also increased in size, and this as well as the smaller mass occupied a more central position. The appearance of these bodies was unlike anything I had previously seen in preparations direct from the tissues.

On the fourth day no doubt remained that a remarkable development was taking place. From Captain Rogers' description I was not prepared for such a great increase in size as occurred, nor for such startling changes in the appearance of the bodies. On this day, although many forms were still unaltered, very many had undergone the change noted above, whilst others had increased four or five fold and, though still retaining the characteristic double chromatin masses, had an appearance totally unlike the original bodies.

Large forms.—The large forms, which had apparently risen each from a single parasite, were fairly abundant and many examples were readily found. In most cases they were as large or larger than the red blood corpuscles.

measuring in some instances as much as $\frac{1}{2}\mu$ in diameter. As a rule, the bodies were slightly oval or bean-shaped. The protoplasm was finely reticular and stained a distinct blue. The chromatin masses still retained their characteristic arrangement and appearance, but were situated well within the protoplasm, instead of being upon the periphery. The large chromatin mass had increased in size and had become granular. In some cases the granules showed a very regular arrangement. The small chromatin mass appeared unaltered or slightly increased in size. It showed most usually the rod-shaped form seen in the original bodies. These forms are undoubtedly those described by Captain Rogers as occurring on the third day, though this observer has not laid very great stress on the extreme enlargement which has taken place in the bodies since their removal from the spleen. No double forms were seen on this day, but commencing fission was already seen in some, an observation not in favour of the conjugation view held by Captain Rogers.

Fifth day.—Large forms resembling those just described were readily found. Bodies with double small and large chromatin masses and commencing fission forms were not uncommon. Most frequently the bodies occurred in groups of two. Groups of four were also seen, and in some instances it was clear that these had arisen from a second fission of groups of two. I could find no evidence that the double forms represented conjugation; it seems much more likely that they are the result of the first fission process.

As these forms lying in groups of two and four have certain characters not seen in later development, I have termed them, for the purpose of description, "primary fission forms."

Primary fission forms.—Though most frequently these forms lie close together in the preparation, they show no trace of the cement substance so characteristic of the later fission forms. In some cases a faintly staining substance retaining the bodies in approximate apposition can be made out, but it is entirely distinct from the red staining substance seen later.

Each form is of large size oval or bean-shaped. A distinct pear shape is not seen at this stage. Fission occurs at least twice before further development of the forms to be described occurs.

The formation of elongate forms.—**One or two forms were seen on the fifth day in which fission had resulted in two rather elongate bodies with the small chromatin masses in opposition to one another and occupying one extremity of the parasite.**

The formation of a vacuole-like area.—**In certain of the forms, apparently after fission had produced at least four separate bodies, a new structure was visible. This structure is not mentioned by Captain Rogers, though it is fairly**

indicated in one or two of his figures. It is seen as a pink staining area about the same size as the large chromatin mass. It lies generally in the neighbourhood of the small chromatin mass. It appears to be of the nature of a vacuole, but contains strands or threads of a material which stains light pink.

In all later stages a proportion of the parasites showed this appearance more or less clearly.

The extrusion of the cement substance.—On the fifth day some curious forms were seen in which a red staining substance of a [illegible] appearance seemed to be poured out from the parasite. This was first noticeable as a tail-like process projecting from the end of the parasite in which the small chromatin mass is situated. In some instances the region of the streak appeared to be in close connection with the small chromatin mass, and in these early stages it might be mistaken for the commencing formation of the flagellum. Later the substance becomes more voluminous and forms are seen in which it appears to be pouring out into the plasma (Fig. 13). The substance stains at first a bright red, but when in large masses it [illegible] a dark red colour. This substance at first is [illegible] very much, and eventually surrounds the end of the parasite in which the small chromatin mass is situated. Afterwards it fuses with that formed by adjoining parasites, and binds the members of a group firmly together (Figs. 15 and 16).

The formation of the flagellum.—[illegible] the fifth day the earliest flagella were [illegible] later. [illegible], however, showed an appearance which may indicate the method of formation of the flagellum. Apart from this, I have not been able to trace any stage of the flagellum prior to its appearance as a complete form. In some of the bodies seen on the fifth day a faintly stained, long, but rather [illegible] filament extended from the end from which the flagellum [illegible] appears. The [illegible] by this filament approximates almost exactly to that of the pink vacuole-like area, and in several instances the protrusion of this delicately staining filament appeared to be connected with the passage of the vacuole to the surface (Fig. 14).

The filament, though clearly distinguishable, was quite unlike the defined [illegible] flagellum which is seen later. It is most probable that the faintly staining filament represents the flagellum when first extruded.

In later forms, when the cement substance has obscured the view, it is very difficult to ascertain what is happening at the end of the parasite containing the small chromatin mass.

Sixth day.—On this day isolated forms were quite exceptional and the bodies were seen lying in groups of from four to ten, groups of four or five being most common. The individual forms were still large and conspicuous.

being rarely less than the size of the red cell. In almost all instances large masses of red staining material had been thrown out.

Most of the bodies were pear-shaped or elongated. The extremities of the parasite containing the small chromatin masses invariably lay close together, whereas the rounded anterior portions projected freely. The pink vacuole was a conspicuous feature in many of the forms, and others possessed distinct, clearly outlined, deeply staining flagella.

Flagellate forms.—On the sixth day almost every group contained one or more flagellate forms. In some cases all the bodies composing the group had developed flagella. Occasionally isolated flagellate forms were seen, but they were not common. The attached end of the flagellum could, as a rule, be traced to the small chromatin mass or to its close neighbourhood. The small chromatin mass thus appears to be a blepharoplast. In many cases it was difficult to trace the flagellum to its origin on account of the cement substance. At the free end of the flagellum a small but distinct knob was very often visible. The length of the flagellum varied, being from 12 μ to 30 μ in length. The flagella, especially the shorter ones, appeared rather stiff and rod-like, though in some cases they had an undulating outline. On the sixth, seventh and eighth days fission produced both large and elongate forms (fig. 16). In some cases the elongate forms were very striking and trypanosome-like; but in no case did the flagellum pass forwards along the large chromatin mass. As a rule, the greatly elongate bodies formed members of groups in which the majority of forms were much less attenuated. In all cases the small chromatin mass lay towards the centre of the groups, and flagella when present passed directly from this end (fig. 16).

Development of bodies included in cells.—On the sixth day and, indeed, throughout the earlier stages it was not unusual to find bodies undergoing development whilst included in the altered substance of a cell. As development progressed, and a group of large forms resulted, these projected so as to give rise to a fungating like mass of the bodies (figs. 20 and 21). The growth of forms which are undoubtedly included in a cell is interesting in relation to the fact that such forms are in the majority in the spleen. It would appear that an included form is not necessarily a dead one.

On this day an unstained drop of the citrated blood was examined, but though several groups of hyaline nucleated bodies were seen no trace of flagella or appearance suggesting motion of these organs could be made out.

Seventh day.—On this day groups composed of many forms were the most common. Most of the groups contained several flagella-bearing individuals. The red staining cement substance was very voluminous and conspicuous. Many

of the bodies showed the pink staining reaction already described. Elongate forms were not more numerous than on the previous day. Fission appeared to be proceeding actively.

Ninth day.—On the ninth day isolated forms were rare and the bodies occurred almost exclusively in large aggregate masses. Elongate forms were exceptional and most of the parasites were pear-shaped or polygonal from mutual pressure. The general cytoplasm was very voluminous, and flagella were sometimes seen made by coalescing rods (fig. 17). Short thick flagella were generally to be seen in a proportion of the individuals of each group. Fission appeared to be taking place more irregularly than heretofore, and difficulty was often experienced in tracing out the life-cycles of individual forms and in assigning to each their respective chromatin masses.

Secondary fission forms.—On the sixth day it was evident that fission of the bodies was proceeding rapidly, and from this day onwards fission, leading to the formation of large and larger masses, composed of bodies of various sizes, was one of the chief features of development. This final condition of repeated fission is not noted by Captain Rogers. It appears to follow, in some cases at least, upon the formation of the gliding pairs, which is the most advanced stage described by this observer. Considerable difference in appearance exists between the large multiple forms which I have described as primary fission forms and the bodies now under discussion. From the ninth day onwards fission seemed to produce smaller and smaller as well as more irregular bodies, which may be conveniently termed secondary fission forms.

Twelfth day.—Subdivision had still further progressed. Individual forms were now small, the majority being larger than the original bodies prior to development. The general appearance of the developed forms was by this time entirely unlike the parasites as drawn from the spleen, and had not all intermediate forms been seen, their identity would scarcely be suspected. Flagella occurred as before in certain individuals only. No changes suggesting degeneration or breaking up of the bodies were seen, and fission still seemed to be in progress. On this day an unstained preparation was examined and a single large group of bodies encountered, but no movement could be made out nor were flagella visible.

Preparations were also examined on the fifteenth and twenty-first days, but the appearances were those seen on the twelfth day, except that groups were more scanty. On the thirty-third day bodies were found with great difficulty, and not only showed no further changes, but appeared to be showing signs of dissolution. As the amount of blood had become very small, no further examinations were made.

On the twelfth day a portion of this culture was removed in a sterile pipette

and added to about double its volume of distilled water, and placed again in the ice chest. A further portion was similarly added to normal salt solution.

Two days later prolonged search failed to reveal any structure resembling the parasites.

On the fifteenth day about half the blood remaining was removed in a pipette and added to another tube, the whole process being very carefully conducted to avoid bacterial contamination. The tube was then enclosed in a moist chamber and incubated at 35°C. On the second day films were examined for the bodies. Only a very few forms all showing marked degenerative changes, bordering on dissolution, were seen. On the fourth day no trace of the parasites could be observed. The preparation was, so far as could be seen under the microscope, free from micro-organisms.

There could be no doubt from this case that, under the conditions described by Captain Rogers, profound developmental changes in the bodies had taken place. These appeared to be of the following nature and order of sequence: enlargement together with changes in the large chromatin mass and the protoplasm, proceeding until the bodies are at least as large as a red blood corpuscle; fission occurring at least twice and forming groups of two and four large oval bodies loosely, or not at all attached; formation of the vacuolar area and extrusion of the cement substance; flagellation of certain of the bodies, and the formation of numerous fission forms many of which flagellate; finally, the formation of large groups of closely aggregate forms of various sizes. The time taken in this case for the different stages was as follows: large fully developed forms appeared by the fourth day; the vacuole, the extrusion of the cement substance, and the early stages of the flagella formation, all first appeared on the fifth day. Fully developed flagella were not seen till the sixth day. Elongate flagellate forms were specially numerous on the sixth and seventh days. From the ninth day onwards the formation of masses of pear-shaped and irregular forms was the chief feature. Captain Rogers notes large forms on the second day and flagellate forms on the third day. He does not note any further stages than those seen on the fourth day, which correspond with those obtained by me on the fifth and sixth days.

Case 2.—The patient had a greatly enlarged spleen and an irregular high temperature. About ·25 c.c. of blood was obtained by spleen puncture. Bodies were rather scanty in a film taken at the time of puncture. In this specimen, after subsidence of the corpuscles, a slight clot formed in the plasma.

On the third day no development was noted. On the fifth day some large forms not quite so large as in the fully developed bodies noted in Case 1 were seen. Most of the bodies had undergone little or no change. On the tenth day

a good many more enlarged bodies were seen, and some large forms at the same stage as those seen on the fifth day. Preparations were not again examined until the twenty-first day, when only one or two undeveloped bodies were noted. In this case development, although started, apparently failed to progress.

Case 3.—The patient had a greatly enlarged spleen and occasional slight rises of temperature. About ·25 c.c. of blood was obtained by splenic puncture. Parasites were fairly numerous in a film taken at the time. On the third day the culture was examined, but only a very few somewhat enlarged bodies were seen. On the tenth day fairly numerous forms with long flagella were seen. In this specimen the bodies were almost entirely free forms; a few groups were, however, present, and two or five bodies were often seen lying close together; a large proportion of the forms possessed flagella four or five times the length of the body of the parasite. The parasites were for the most part of an elongate pear-shaped form, and in many cases the extremity opposite to the flagellum was pointed, whereas the extremity from which the flagellum arose represented the thick end of the pear. In these forms, as in all those I have seen, the flagellum passed directly outwards from the small chromatin mass. A good many individuals showed small chromatin particles in addition to the two main masses. There were some forms arranged in a [illegible] of the [illegible]. In some of the more elongate forms there appeared to be large chromatin masses irregularly dispersed, so that it was difficult to say which mass represented the macronucleus. Captain Rogers figures a similar condition in some of his forms.

In this case development had apparently differed somewhat in its nature from that in case 1. This variability would appear also to have occurred in Captain Rogers' cultures, which had [illegible] flagellate forms on the first day. In this connection, it is worthy of note that in some cultures, although sterile, a much darker colour of the blood was seen than in others. It is therefore possible that all cultures may not be under precisely similar conditions.

Case 4.—The patient had a greatly enlarged spleen and an irregular high temperature. About ·5 c.c. of blood was drawn off by splenic puncture and placed in two tubes. The tubes were placed direct into a cool incubator at 22°C. On the fifth day some large characteristic bodies were seen resembling those seen in Case 1. The specimen was not examined later.

Although I have examined only a small number of cases, the results have been amply sufficient to corroborate the changes described by Captain Rogers. But, although the presence of a nucleus and well-marked blepharoplast and the development of a flagellum point to the bodies being a flagellate, I do not feel justified, either from my own observations or from an inspection of Captain Rogers'

drawings, in stating that they are *trypanosomata*. The relation of the flagellum to the large chromatin mass, even in the very elongate forms, does not suggest that the characteristic arrangement met with in trypanosomes is in process of development. On the other hand, it cannot be denied that the forms bear a very close resemblance to some of the developmental forms seen in cultures of *T. lewisi*, and further study must determine what relation, if any, exists between the new parasite and the *trypanosomata* of mammals.

EXPLANATION OF PLATE.

Fig. 1. Bodies as seen at times made direct from splenic blood

Figs. 2, 3 and 4. Forms seen prior to 3rd day on cultivated blood. Case 1.

Fig. 5. First unmistakable developmental change, 3rd day. Case 1.

Fig. 6. Form which has developed to full size, 4th day. Case 1.

Figs. 7, 8 and 9. Primary division forms seen on 4th and 5th day. Fig. 9 shows a group of four such forms. Case 1.

Fig. 10. Bodies forming a group held together by a light staining substance, 5th day. Case 1.

Fig. 11. Elongate forms with the small chromatin mass at one extremity, 5th day. Case 1.

Fig. 12. Group of four bodies showing between the first appearance of the flagellum and the formation of the vacuole, 6th day. Case 1.

Fig. 13. Bodies showing first appearance of the cement substance, 6th day. Case 1.

Figs. 14, 15. Groups of flagellate forms seen on the 6th day. Case 1.

Fig. 16. Group of bodies showing elongate flagellate forms, 6th day. Case 1.

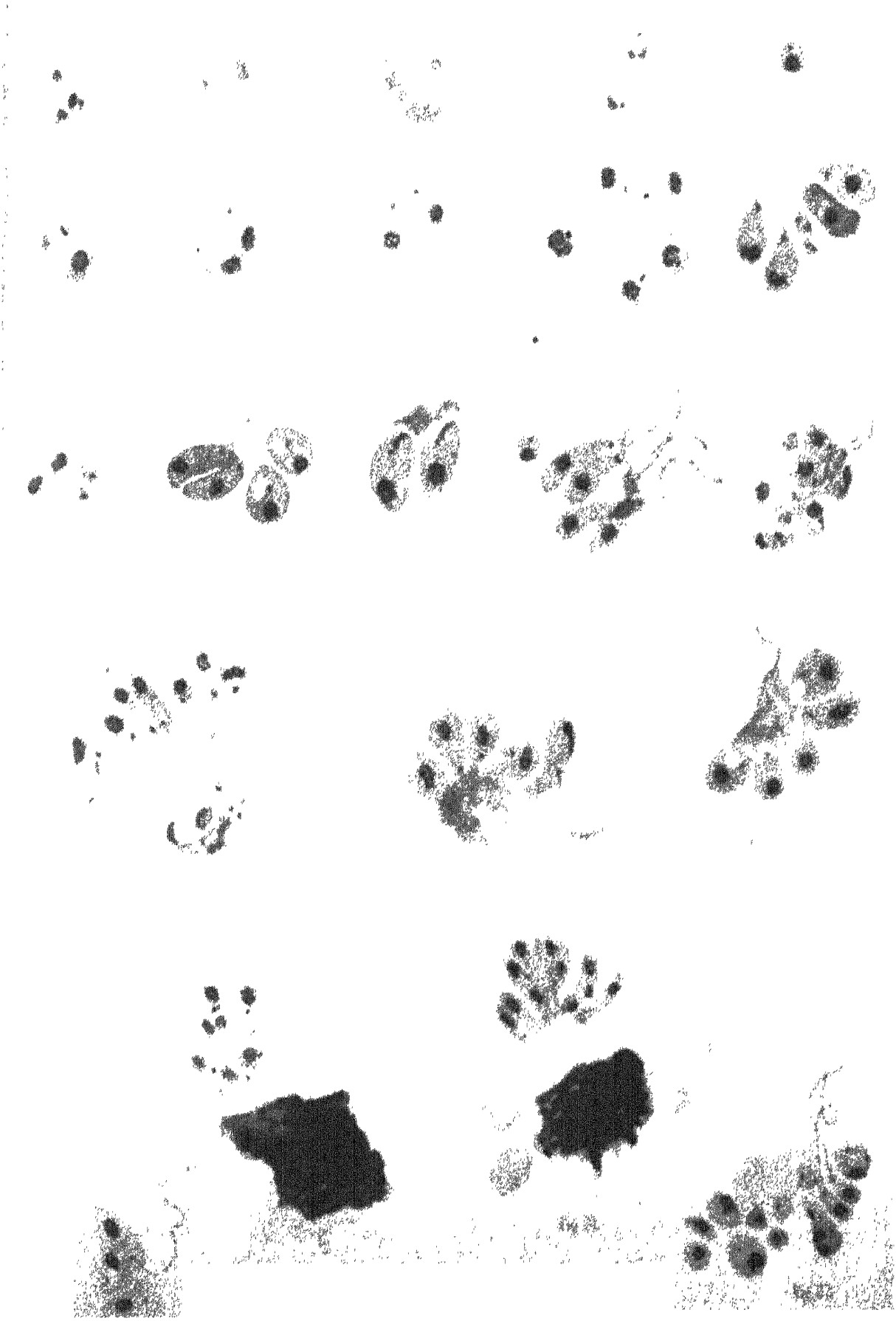
Figs. 17, 18. Groups of bodies bound together by voluminous cement substance in which flagella are seen enmeshed. Case 1.

Fig. 19. Form showing long flagellum. Case 1.

Figs. 20 and 21. Groups of bodies which appear to have arisen from forms included in 17.

Fig. 22. Group of crescentic forms, showing cement substance, small forms, irregular subdivision and flagellate bodies, 12th day. Case 1.

The changes seen after the twelfth day are not figured.



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ON A PARASITE FOUND IN PERSONS SUFFERING FROM ENLARGEMENT OF THE SPLEEN IN INDIA.—(THIRD REPORT.)

BY

LIEUT. S. R. CHRISTOPHERS, M.B., I.M.S.

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